Application Data Sheet (ADS) with amendment to the priority claims; and 5) An Abstract. Applicant also encloses a copy of Provisional Application 60/015,869, filed May 31, 1996, as requested by the Examiner.

Reconsideration of this application and pending claims in view of the amendments and discussion below is respectfully requested.

IN THE SPECIFICATION

Please enter the amendments to the specification below, the amendments of which are provided in Appendix I.

1. Please delete the Preliminary Amendment filed on August 27, 1999 and replace with the following paragraph at page 1, line 5 of the specification:

E1 This invention was made with government support under Contract Nos. CA50826, CA45726, HL54444, T32 AI07244-11 and F32 CA72192 by the National Institutes of Health. The government has certain rights in the invention.

2. At page 8, lines 10-21, please insert the amended paragraph:

Figures 15A, 15B, 15C and 15D show the consecutive cDNA sequence of chicken MMP-2 along with the deduced amino acid sequence shown on the second line, as shown in Figures 15A, 15B and 15C. The third and fourth lines respectively show the deduced amino acid sequence of human and mouse MMP-2 as described in Example 7. The chicken cDNA sequence is listed in SEQ ID NO 23 along with the encoded amino acid sequence that is also presented separately as SEQ ID NO 24. The numbering of the first nucleotide of the 5' untranslated region and region encoding the proenzyme shown in Figure 15A as a negative number is actually presented as number 1 in Sequence Listing making the latter appear longer than the figure; however, the nucleotide sequence is the figure is identical in length and sequence to that as presented in the listing with the exception of the numbering. Accordingly, references to

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E2 nucleotide position for chicken or human MMP-2 in the specification, such as in primers for use in amplifying MMP-2 fragments, are based on the nucleotide position as indicated in the figure and not as listed in the Sequence Listing.

3. At page 42, lines 1-12, please insert the amended paragraph:

** The human MMP-2 amino acid residue sequences for synthetic peptides are indicated by the corresponding residue positions shown in Figures 15A - 15C and also in Figure 16. (MMP-2 refers to a member of the family of matrix metalloproteinase enzymes). The human MMP-2 sequences are listed with the natural cysteine residues but are not listed with engineered cysteine residues as described for the fusion peptides. The non-natural cysteine residues were substituted for the natural amino acid residue at the indicated residue positions in order to facilitate solubility of the synthetic as well as expressed fusion proteins and to ensure proper folding for presentation of the binding site.

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*** The chicken MMP-2 amino acid residue sequences for synthetic peptides are indicated by the corresponding residue positions shown in Figures 15A - 15C. The chicken MMP-2 sequences are listed with the natural cysteine residues but not with the engineered cysteine residues as described for the fusion peptides as described above.

4. At page 55, lines 12-25, please insert the amended paragraph:

The chicken-derived MMP-2 C-terminal domain, also referred to as the hemopexin domain immediately contiguous with the hinge region, comprises the amino acid residues 445-637 of MMP-2. The complete nucleotide and encoded amino acid sequence of chicken MMP-2 is described below and is shown in Figures 15A - 15D, with the nucleotide and amino acid sequences respectively listed as SEQ ID NOs 23 and 24. The human MMP-2 nucleotide and encoded amino acid sequence is also described below, with the latter shown in Figure 16 and SEQ ID NO 25. The C-terminal domain in the human MMP-2 that corresponds to the chicken region of 445-637 begin at amino acid residue 439 and ends with 631 due to six missing residues from the

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human sequence as shown in Figure 15C. Both human- and chicken-derived C-terminal MMP-2 synthetic peptides for use in practicing the methods of this invention are listed in Table 1. The amino acid residue sequences of the synthetic peptides are the same as those generated by the recombinant fusion protein counterparts but without the GST fusion component. The C-terminal MMP-2 fusion proteins derived from both chicken and human are prepared as described below.

5. At page 55, beginning at line 29, continuing to page 56, lines 1-12, please insert the amended paragraph:

To amplify various regions of chicken and human MMP-2, primer sequences were designed based on the known respective cDNA sequences of chicken and human MMP-2. The complete top strand of the cDNA nucleotide sequence of unprocessed chicken MMP-2, also referred to as progelatinase, is shown in Figures 15A - 15D along with the deduced amino acid sequence shown on the second line (Aimes et al., Biochem. J., 300:729-736, 1994). The third and fourth lines of the figure respectively show the deduced amino acid sequence of human (Collier et al., J. Biol. Chem., 263:6579-6587 (1988)) and mouse MMP-2 (Reponen et al., J. Biol. Chem., 267:7856-7862 (1992)). Identical residues are indicated by dots while the differing residues are given by their one letter IUPAC lettering. Missing residues are indicated by a dash. The numbering of the amino acid residues starts from the first residue of the proenzyme, with the residues of the signal peptide being given negative numbers. The nucleotide sequence is numbered accordingly in the figure although in the Sequence Listing, the first nucleotide is listed as number 1. The putative initation of translation (ATG) is marked with three forward arrowheads and the translation termination signal (TGA) is indicated by an asterisk. The amino terminal sequences for the chicken proenzyme and active enzyme are contained with diamonds and single arrowheads. As previously stated, the chicken progelatinase nucleotide and amino acid residue sequences are listed together as SEQ ID NO 23 while the encoded amino acid residue sequence is listed separately as SEQ ID NO 24.

6. At page 56, beginning at line 27, continuing to page 57, lines 1-11, please insert the amended paragraph:

From either of the above-described cDNA templates, a number of C-terminal regions of chicken MMP-2, each having the natural cysteine residue at position 637 at the carboxy terminus, were obtained by PCR with the 3' primer listed above (SEQ ID NO 26) paired with one of a number of 5' primers listed below. The amplified regions encoded the following MMP-2 fusion proteins, having sequences corresponding to the amino acid residue positions as shown in Figures 15B and 15C and also listed in SEQ ID NO 24: 1) 203-637; 2) 274-637; 3) 292-637; 4) 410-637; 5) 445-637. Upstream or 5' primers for amplifying each of the nucleotide regions for encoding the above-listed MMP-2 fusion proteins were designed to encode the polypeptide start sites 3' to an engineered, i.e., PCR-introduced, internal BamHI restriction site to allow for directional ligation into either pGEX-1λT or pGEX-3X expression vectors. The 5' primers included the following sequences, the 5' and 3' ends of which correspond to the indicated 5' and 3' nucleotide positions of the chicken MMP-2 sequence shown in the figure (the amino acid residue position start sites are also indicated for each primer): 1) Nucleotides 599-619, encoding a 203 start site 5'ATGGGATCCACTGCAAATTTC3' (SEQ ID NO 27); 2) Nucleotides 809-830, encoding a 274 start site 5'GCCGGATCCATGACCAGTGTA3' (SEQ ID NO 28); 3) Nucleotides 863-883, encoding a 292 start site 5'GTGGGATCCCTGAAGACTATG3' (SEQ ID NO 29); 4) Nucleotides 1217-1237, encoding a 410 start 5'AGGGGATCCTTAAGGGGATTC3' (SEQ ID NO 30); and 5) Nucleotides 1325-1345, encoding a 445 start site 5'CTCGGATCCTCTGCAAGCACG3' (SEQ ID NO 31).

7. At page 58, beginning at line 19, continuing to page 59, lines 1-4, please insert the amended paragraph:

Briefly, the pGEX-3X plasmid construct encoding the recombinant GST/MMP-2(410-637) fusion protein prepared above was used as a template for amplification according to the manufacturer's protocol for the Expand High Fidelity PCR Kit